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PLANT GROWTH PROMOTING CHARACTERISTICS OF NON-*RHIZOBIAL* STRAINS ISOLATED FROM ROOT NODULES OF *VIGNA TRILOBATA* CULTIVARS

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ABSTRACT

Rhizobacterial strains were isolated from root nodules of Vigna trilobata plants raised in soils collected from four geographically different areas in A.P. India. The strains were identified by 16Sr DNA analysis as Bacillus altitudinis MRR 122 (KF 621021), Enterobacter cloacae MRR 127 (KF 621020), Mycobacterium wolinskyi MRR 120 (KF 621019) and Paenibacillus sp. strain MRR 124 (KF 621017). These are the first reported non rhizobial strains associated with root nodules of V. trilobata. The strains were biochemically characterized and plant growth promoting activities (PGP) viz., IAA, EPS, Siderophore and HCN production were studied. All the four non rhizobial strains showed IAA and EPS production. Among them maximum IAA production was observed in Bacillus altitudinis MRR 122 (35µg/ml) followed by Enterobacter cloacae MRR 127 (30 µg/ml). Paenibacillus sp. strain MRR 124 and Mycobacterium wolinskyi MRR 120 produced 27 µg/ml and 25 µg/ml of EPS respectively. All the strains produced copious amount of EPS in the range of 631-690 mg/ml. Only two strains, Bacillus altitudinis MRR 122 and Paenibacillus sp. MRR 124 showed positive results for siderophore and HCN production. From the results it is evident that non rhizobial strains Bacillus altitudinis and Paenibacillus sp. from root nodules of V. trilobata, with better PGP activities can be exploited as bioinoculants for biofertilizer production.

KEYWORDS: PGPR, Indole Acetic Acid & Exo Polysaccharide

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Original Article

INTRODUCTION

The nitrogen-fixing bacteria nodulating roots of leguminous plants are collectively known as *rhizobia* and the plant beneficial bacteria colonizing root surfaces and adhering soil interface are known as plant growth-promoting rhizobacteria (PGPR). Root nodules also accommodate various non-*rhizobial* bacteria having definite influence on the survival, nodulation and grain yield of the crop (Remans et al., 2008). This influence may be passive, but most often non-*rhizobial* bacteria synergistically act with *rhizobia* and enhance nodulation and grain yield possibly by Indole Acetic Acid (IAA) production, phosphate solubilization, fixing nitrogen, siderophore production, etc. (Mishra et al., 2009; Rajendran et al., 2008).Therefore, use of non-rhizobial root-nodule bacteria has been increased over the years in order to increase the competitive survivability of *rhizobial* biofertilizers and thus achieve better plant growth under adverse environmental condition (Rajendran et al., 2012).

Vigna trilobata commonly called as 'Pillipesara' was mainly cultivated as short term pasture and green manure crop in India, Indonesia, Pakistan and Sudan. A perusal of literature on *Vigna-Rhizobium* interactions reveals that the studies on nodulation, isolation, cultural and biochemical studies were carried out mainly on few species of *Vigna* viz. *V. mungo, V. unguiculata and V. radiata*. The studies on cultural and biochemical characterization of the *Rhizobium* spp. associated with *V. trilobata* were very merge. There were no reports of non

rhizobial strains associated with root nodules of *V. trilobata* and their importance so far. Hence in the present study, plant growth promoting characteristics like IAA, EPS, Siderophore and HCN production were screened for the non rhizobial strains isolated from root nodules of *V. trilobata*. Effective strains can be employed as inoculants for bio fertilization, Phytostimulation and biocontrol.

MATERIAL AND METHODS

Isolation

Rhizobacteria associated with the root nodules of *Vigna trilobata* plants maintained properly in the botanical garden of our university. For the isolation pink coloured healthy root nodules were collected by gently uprooting the plants, twenty one days after sowing, surface sterilized with 0.1% mercuric chloride and washed several times with sterile distilled water. Bacterial suspension was prepared by crushing these nodules with sterile glass rod using sterile distilled water. A loopful of suspension was spared on media plates containing selective medium yeast extract mannitol agar medium (YEMA) with 0.1% Congo red and incubated at room temperature for 3 days. After incubation, the white translucent, convex, colonies with high mucilage were isolated and pure cultures were maintained using the same medium. Biochemical tests (Somasegaran and Hoben, 1994), and nodulation ability test on homologous hosts by plant infection tests (Vincent, 1970) were conducted for all the strains. On molecular characterization through 16S rDNA sequencing (Macrogen, South Korea) the strains were identified as *Bacillus altitudinis* MRR 122, *Enterobacter cloacae* MRR 127, *Paenibacillus* sp. MRR 124 and *Mycobacterium wolinskyi* MRR 120. These sequences were deposited in the Gene bank. All the four strains were screened for their potential for the production of plant growth promoting traits like IAA, EPS, Siderophore and HCN production.

IAA Production

All the four non *Rhizobial* strains were screened for IAA production (Gordon and Weber, 1951) by inoculating them into 100 ml conical flaks containing YEM broth supplemented with L-tryptophan. The flasks were incubated at 30 ± 1 °C on rotary shaker (150 rpm) for 72 hours. After incubation medium was centrifuged at 5000 x g for 20 minutes and cell free supernatant was used for IAA extraction23. To 10 ml of supernatant, 2 ml Salkowski reagent was added and incubated for 30 minutes under darkness. Amount of IAA produced was determined calorimetrically at 540 nm.

EPS Production

For the estimation of EPS production, all the four strains were inoculated separately into Erlenmeyer flasks (250 ml) containing 100 ml YEM broth supplemented with 1% Mannitol. The flasks were Incubate at RT on orbital shaker at 200 rpm for 72 h. After incubation the broths were centrifuged at 3000 x g and the supernatant was mixed with 2 volumes of chilled acetone. The crude polysaccharide precipitate was collected by centrifugation at 3000 x g for few minutes. The EPS was washed with distilled water and acetone alternately, transferred into a filter paper and weighed after overnight drying at 105^0C (Damery and Alexander, 1969).

Siderophore Production

Siderophore production was detected by the universal method of Schwyn and Neilands (1987) using blue agar plates containing the dye chromo azurol S (CAS). Orange halos around the colonies on blue were indicative for siderophore production.

HCN Production

HCN Production was determined by the method of Miller and Higgins (1970) with slight modifications. Actively growing bacterial cultures were streaked on YEM plates supplemented with 4.4 g/l glycine with simultaneous supplementation of a filter paper soaked in 0.5% Picric acid in 1 % Na_2CO_3 in the upper lid of petri plates. The plates were sealed with parafilm. Control plates didn't receive inoculum. The plates were incubated for seven days at room temperature. Change in colour of the filter paper from yellow to light brown, moderate brown or strong reddish brown indicates the HCN production.

RESULTS AND DISCUSSIONS

All the four strains were screened for their plant growth promoting activities viz. Indole Acetic Acid production, EPS production, Siderophore and HCN production. The results showed that not all the strains exhibited all the four PGP activities. The positive strains for each of PGP activity varied greatly (Table-1). There were no previous records on *Mycobacterium wolinskyi* and *Enterobacter cloacae*, isolated from root legume root nodules, exhibiting plant growth promoting traits.

Table 1: Plant Growth Promoting Characteristics of Non-*Rhizobial* Strains from *Vigna Trilobata*

S. No.	Strain Name	PGPR			
		IAA	EPS	Siderophore	HCN
1	*Bacillus altitudinis* MRR 122	+	+	-	+
2	*Enterobacter cloacae* MRR 127	+	+	-	-
3	*Mycobacterium wolinskyi* MRR 120	+	+	-	-
4	*Paenibacillus* sp. MRR 124	+	+	-	+

IAA Production:

IAA is a plant growth regulating hormone which controls plant cell division and root elongation (Kravchenko et al., 2004). Plant roots release nutrients that contain tryptophan which can be consumed by soil bacteria as a precursor for IAA production, a widespread trait among most plant growth-promoting bacteria. In the present study maximum IAA production of 35µg/ml was recorded in *Bacillus altitudinis* MRR 122. Zhaeo et al (2015) reported that *B. atrophans* an endophyte isolated from *Lonicera japonica* produced 49.2 mg/L of IAA. *B. subtilis* from mung been root nodules produced 28.3 µg/ml of IAA (Tariq et al., 2012). *Paenibacillus* sp. MRR 124 strain produced 27 µg/ml however previous reports indicate that *P. taichungensis* from *Vigna radiata* produced only 10.8 µg/ml of IAA (Pandya et al., 2015). *Mycobacterium wolinskyi* MRR 120 and *Enterobacter cloacae* MRR 127 produced 25 µg/ml 30 µg/ml of IAA respectively, in presence of 1% L- tryptophan after 72 hours of incubation (Figure 1).

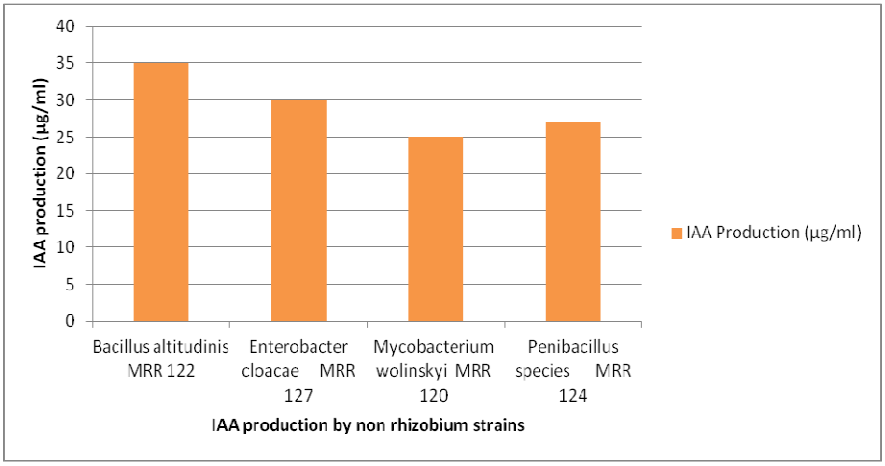

Figure 1: Indole Acetic Acid Porduction (µg/ml) by Non Rhizobial Strains Isoalted from Root Nodules of *V. Trilobata*

EPS Production

Among the four strains, *Enterobacter cloacae* MRR 127 produced maximum EPS of 690 mg/ml. However, Mane and Hamde (2015) reported that *Enterobacter cloacae* isolated from root nodules of *Cicer arietinum* produced only 500µg/ml of EPS. *Enterobacter* sp isolated from root nodules of *Abrus precatorius* produced 122 µg /ml of EPS was reported by Ghosh et al (2016). *Enterobacter ludwigii* isolated from *Medicago lupulina* root produced 0.7g/L of EPS (Pawlikijullian et al., 2010) indicating that there is strain difference in the EPS production. Similar to *Enterobacter cloacae* MRR 127, *Bacillus altitudinis* MRR 122 produced 686 mg/ml of EPS. *Mycobacterium wolinskyi* MRR 120 and *Paenibacillus* sp. MRR 124 produced slightly low amount of EPS as 656 mg/ml and 631 mg/ml respectively (Figure 2).

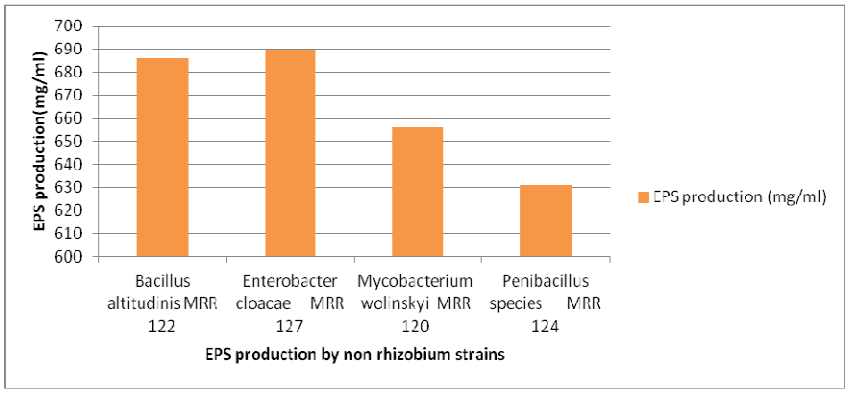

Figure 2: Exopolysacharide Production (mg/ml) by Non Rhizobial Strains Isoalted from Root Nodules of *V. Trilobata*

Paenibacillus spp. produced a wide variety of different EPSs with diverse physiological and biotechnological functions. Previous reports indicate that *Paenibacillus maracerans TKU029* produced 3.46g/L of EPS (Liang et al., 2014) however the species in this study *Paenibacillus* sp. MRR 124 produced 631 mg/ml of EPS.

Siderophore and HCN Production

Siderophore production is an essential PGP trait possessed by majority of *rhizobacteria* for iron uptake in the Rhizosphere. Siderophores bind to the available form of Fe^{3+}thus making it unavailable to the competing phytopathogens

and protecting the plant. Two out of four non *rhizobium* strains *Bacillus altitudinis* MRR 122 and *Paenibacillus* sp. MRR 124 are positive for siderophore and HCN production.

Pandya et al (2015) reported that the plant growth promoting traits of gram positive *Bacillus* and *Paenibacillus* sp. were well known for siderophore production. They reported that *Bacillus anthrasis* isolated from *Vigna radiata* produced 46.77 µg/ml of siderophore.

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CONCLUSIONS

This was the first report of studies on non rhizobial isolates from root nodules of vigna trilobata. Enterobacter cloacae MRR 127 and Bacillus altitudinis MRR 122 were porved to be best isolates with best PGPR characters of maximum EPS and IAA production.

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